

AMENDMENT TO THE CLAIMS

LISTING OF CLAIMS:

1. (Original) An isolated nucleic acid molecule comprising a sequence of nucleotides encoding a murine T-type calcium channel α_1H subunit selected from the group consisting of:

(a) a sequence of nucleotides that encodes a murine T-type calcium channel α_1H subunit and comprises the sequence of nucleotides set forth in one of SEQ ID NOS:1 or 5;

(b) a sequence of nucleotides having at least 95% sequence identity or is exactly complementary to the nucleotide sequence set forth in SEQ ID NO:1 or 5, and

(c) a nucleotide sequence varying from the nucleotide sequence specified in (a) or (b) as a result of degeneracy of the genetic code.

2. (Original) A substantially pure polypeptide comprising an amino acid sequence selected from the group consisting of: (i) an amino acid sequence coded by the isolated nucleic acid molecule of claim 1; (ii) homologues of the amino acid sequences of (i) in which one or more amino acids has been added, deleted, replaced or chemically modified in the region, or adjacent to the region, where the amino acid sequences differs from the original amino acid sequence, coded SEQ ID NOS: 1 or 5.

3. (Original) A substantially pure polypeptide comprising an amino acid sequence encoded by the nucleotide sequence as set forth in one of SEQ ID NOS: 1 or 5.

4. (Original) A substantially pure polypeptide comprising an amino acid sequence as set forth in one of SEQ ID NOS: 2 or 6.

5. (Original) An expression vector comprising the nucleic acid molecule of claim 1 operably linked to a regulatory nucleotide sequence that controls expression of the nucleic acid molecule in a suitable host cell.

6. (Original) A recombinant host cell transfected by the expression vector of claim 5.

7. (Original) A method for detecting the presence of a nucleic acid sequence of $\alpha 1H$ in a biological sample, comprising the steps of: (a) hybridizing to nucleic acid material in said biological sample the nucleic acid molecule of claim 1 under conditions favoring the formation of a hybridization complex; and (b) detecting said hybridization complex; wherein the presence of said hybridization complex correlates with the presence of an variant nucleic acid sequence in the said biological sample.

8. (Original) A method for determining the level of a nucleic acid sequences of $\alpha 1H$ subunit or a variant thereof in a biological sample comprising the steps of: (a) hybridizing to nucleic acid material of said biological sample the nucleic acid sequences of claim 1; and (b) determining the amount of hybridization complexes and normalizing said amount to provide the level of the $\alpha 1H$ subunit or variant thereof encoding nucleic acid sequences in the sample.

9. (Amended) A method for detecting the level of the polypeptide variant of ~~SEQ ID NO:2 or 6~~ claim 4 or a biologically active fragment or variant thereof in a biological sample, comprising the steps of: (a) contacting said biological sample with a detectable antibody having binding specificity for a polypeptide of SEQ ID NO: 2 or 6, thereby forming an antibody-polypeptide complex; and (b) detecting the amount of said antibody-polypeptide complex and normalizing said amount to provide the level of said amino acid sequence in the sample.

10. (Original) A method for identifying lead compounds for a pharmacological agent useful in the treatment of disease associated with increased or decreased voltage regulated calcium influx mediated by a rat T-type calcium channel comprising:

(i) providing a cell expressing a rat T-type calcium channel subunit polypeptide designated herein as $\alpha 1H$; said calcium channel subunit comprising the amino acid sequence as set forth in one of SEQ ID NOS: 2, 4 or 6;

(ii) contacting the cell with a candidate pharmacological agent under conditions which, in the absence of the candidate pharmacological agent, to thereby cause a first amount of voltage regulated calcium influx into the cell; and

(iii) determining a test amount of voltage regulated calcium influx as a measure of the effect of the lead compounds for a pharmacological agent on the voltage regulated calcium influx mediated by a human T-type calcium channel, wherein (a) the test amount of voltage regulated calcium influx which is less than the first amount indicates that

the candidate pharmacological agent is a lead compound for a pharmacological agent which reduces voltage regulated calcium influx and (b) wherein a test amount of voltage regulated calcium influx which is greater than the first amount indicates that the candidate pharmacological agent is a lead compound for a pharmacological agent which increases voltage regulated calcium influx.

11. (Original) The method of claim 10, further comprising loading said cell with a calcium-sensitive dye which is detectable in the presence of calcium, wherein the calcium-sensitive dye is detected as a measure of the voltage regulated calcium influx.

12. (Amended) A method for identifying compounds which selectively bind a T-type calcium channel α_1H subunit comprising, (i) providing a test cell preparation, wherein said cell expresses a polypeptide encoded by the nucleic acid molecule of claim 1 ~~a rat T-type calcium channel α_1H subunit~~, (ii) providing a control cell preparation, wherein said cell expresses a rat T-type calcium channel non- α_1H subunit, with the proviso that the cell in the control cell preparation is identical to the test cell except for the expression of a non- α_1H being expressed, (iii) contacting the test cell preparation and the control cell preparation with a compound, and (iv) determining the binding of the compound to the test cell preparation and the control cell preparation, wherein a compound which binds the test cell preparation but does not bind the control cell preparation is a compound which selectively binds the a mammalian T-type calcium channel α_1H subunit.

13. (Original) A diagnostic method for predicting an oncogenic potential of a sample of cells, comprising:

(a) determining, in the sample levels of expression of a target gene sequence as claimed in claim 8 and comparing said sequence with the sequence as set forth in GenBank Accession No. AF290213 to determine mutations in the target sequences or its complement, wherein excessive or insufficient levels of expression of said target sequence relative to normal is predictive of the oncogenic potential of said cells.

14. (Original) The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is cDNA.

15. (Amended) A method of producing the recombinant protein according to claim 3 ~~or 4~~, comprising:

- (a) inserting the nucleic acid sequence as set forth in SEQ ID NO: 1, 3 or 5 or a fragment or variant thereof into an expression vector;
- (b) transferring the expression vector into a host cell; or transfecting or transforming a host cell with the expression vector of step (a) above;
- (c) culturing the host organism under conditions appropriate for amplification of the vector and expression of the protein; and
- (d) harvesting the recombinant protein from the culture.

16. (Amended) A method for identifying compounds that modulate the activity of the polypeptide of claim 3 ~~a T-type calcium channel α_1H subunit~~, the method comprising:

comparing the difference in the amount of transcription of a reporter gene in a cell in the presence of the compound with the amount of transcription in the absence of the compound, or with the amount of transcription in the absence of a heterologous T-type calcium channel α_1H subunit, whereby compounds that modulate the activity of the heterologous calcium channel subunit in the cell are identified, wherein the cell comprises a nucleic acid molecule that encodes a reporter gene construct containing a reporter gene in operative linkage with one or more transcription control elements that is regulated by a calcium channel and furthermore the cell is a eukaryotic cell transfected with a nucleic acid molecule comprising the coding portion of the sequence of nucleotides set forth in one of SEQ ID NO: 1 or 5.

17. (Amended) A method for identifying a test compound capable of modulating the activity of the polypeptide of claim 3 ~~T-type calcium channel α_1H subunit~~, the method comprising :

- (i) suspending a eukaryotic cell in a solution containing the compound and a calcium channel selective ion;
 - (ii) depolarizing the cell membrane of the cell, and
 - (iii) detecting the current or ions flowing into the cell,
- wherein the eukaryotic cell comprises a functional calcium channel that contains at least one subunit encoded by a heterologous nucleic acid comprising the coding portion of the sequence of nucleotides set forth in SEQ ID NOs: 1 or 5, and
- wherein the current that is detected is different from that produced by depolarizing the same or a substantially identical cell in the presence of the same calcium channel selective ion but in the absence of the test compound.

18. (Original) The method of claim 17, wherein prior to the depolarization step the cell is maintained at a holding potential which substantially inactivates calcium channels that are endogenous to the cell.

19. (Amended) A method for determining whether a test compound inhibits calcium channel activity in cells, said method comprising:

(a) culturing recombinant cells expressing a functional calcium channel including as a component a functional polypeptide according to claim 3 ~~T-type calcium channel α_1H subunit~~ under conditions where intracellular calcium concentrations depend on calcium channel activity; and

(b) measuring intracellular calcium concentrations in the cultured recombinant cells in the presence and absence of the test compound to determine whether the intracellular calcium concentration in the recombinant cells in the presence of the test compound is lower than the intracellular calcium concentration in the cells cultured in the absence of the test compound, wherein a test compound which lowers said calcium concentration is considered to be a calcium channel inhibitor.

20. (Original) A method as in claim 19, wherein intracellular calcium concentration is measured by observing a change in fluorescence of a calcium sensitive dye which is introduced to the cultured recombinant cells prior to the test compound.